

WHAT IS CLAIMED IS:

1. (currently amended) A biofuel cell for generating electricity using a fuel fluid comprising:

a non-conducting substrate;

a cathode supported by the substrate and capable of a reaction to reduce an oxidant in the presence of electrons to form water;

an anode supported by the substrate and capable of a reaction to oxidize the fuel fluid;

at least one of the anode and cathode including an enzyme for use in carrying out its respective reaction; and either

(i) at least one of the anode and cathode being formed for flow of the fuel fluid therewithin for use in producing an electrical current; or

~~(ii) the cathode comprising an enzyme immobilization material comprising a micellar or inverted micellar structure; or~~

(ii) ~~(iii)~~ at least one of the anode and cathode comprising a width less than about 200 μm and at least one surface having an irregular, three dimensional topography capable of inducing convective flow of the fuel fluid and/or oxidant over said surface.

2. (previously presented) The biofuel cell of claim 1, wherein the anode comprises

(a) an electron conductor;

(b) an electron mediator, the reduced form of the electron mediator being capable of releasing electrons to the electron conductor;

(c) at least one anode enzyme capable of reacting with the oxidized form of the electron mediator and the fuel fluid to produce an oxidized form of the fuel fluid and a reduced form of the electron mediator;

(d) an enzyme immobilization material capable of immobilizing and stabilizing the enzyme, the material being permeable to the fuel fluid and the electron mediator, and optionally, the material comprises the electron mediator.

3. (cancelled).

4. (previously presented) The biofuel cell of claim 1, wherein the anode comprises

(a) an electron conductor;

(b) an electron mediator;

(c) at least one enzyme capable of reacting with the oxidized form of the electron mediator and the fuel fluid to produce an oxidized form of the fuel fluid and a reduced form of the electron mediator;

(d) an enzyme immobilization material optionally comprising the electron mediator and/or an electrocatalyst, the material being capable of immobilizing and stabilizing the enzyme, the material being permeable to the fuel fluid; and

(e) an electrocatalyst adjacent the electron conductor, an oxidized form of the electrocatalyst being capable of reacting with the reduced form of the electron mediator to produce an oxidized form of the electron mediator and a reduced form of the electrocatalyst, the reduced form of the electrocatalyst being capable of releasing electrons to the electron conductor.

Claims 5. - 6. (cancelled).

7. (previously presented) The biofuel cell of claim 2 wherein the anode's electron mediator comprises pyrroloquinoline quinone (PQQ), phenazine methosulfate, dichlorophenol, indophenol, short chain ubiquinones, or potassium ferricyanide.

Claims 8. - 10. (cancelled).

11. (previously presented) The biofuel cell of claim 2 wherein the anode enzyme comprises an oxidoreductase that acts on the CH-OH group or CH-NH group, a dehydrogenase, alcohol dehydrogenase, aldehyde dehydrogenase, formate

dehydrogenase, formaldehyde dehydrogenase, glucose dehydrogenase, glucose oxidase, lactic dehydrogenase, lactose dehydrogenase, pyruvate dehydrogenase, or a PQQ-dependent dehydrogenase.

12. (cancelled).

13. (previously presented) The biofuel cell of claim 1, wherein the cathode comprises

(a) an electron conductor;

(b) at least one enzyme capable of reacting with a reduced form of an electron mediator and an oxidant to produce an oxidized form of the electron mediator and water; and

(c) an enzyme immobilization material comprising either the electron mediator, an electrocatalyst, or the electron mediator and an electrocatalyst, the enzyme immobilization material being capable of immobilizing and stabilizing the enzyme, the material being permeable to the oxidant, an oxidized form of the electrocatalyst being capable of gaining electrons from the electron conductor to produce a reduced form of the electrocatalyst that is capable of reacting with an oxidized form of the electron mediator to produce a reduced form of the electron mediator and an oxidized form of the electrocatalyst.

14. (previously presented) The biofuel cell of claim 1, wherein the cathode comprises

(a) an electron conductor;

(b) at least one enzyme capable of reacting with a reduced form of an electron mediator and an oxidant to produce an oxidized form of the electron mediator and water; and

(c) an enzyme immobilization material optionally comprising the electron mediator, the enzyme immobilization material being capable of immobilizing and stabilizing the enzyme, the material being permeable to the oxidant, an oxidized form of

the electron mediator being capable of gaining electrons from the electron conductor to produce a reduced form of the electron mediator.

Claims 15. - 16. (cancelled).

17. (previously presented) The biofuel cell of claim 12 wherein the enzyme comprises a laccase, an oxidase, a glucose oxidase, an alcohol-based oxidase, a cholesterol-based oxidase, an oxygen oxidoreductase, or a bilirubin oxidase.

Claims 18. - 33. (cancelled).

34. (previously presented) The biofuel cell of claim 1 wherein the anode and/or the cathode is formed for flow and the fuel fluid and/or the oxidant is moved through the biofuel cell at a flow rate of between about 0.01 $\mu\text{L}/\text{min}$ and about 10 $\mu\text{L}/\text{min}$.

Claims 35. - 37. (cancelled).

38. (previously presented) The biofuel cell of claim 2 wherein the enzyme immobilization material immobilizing the anode and/or cathode enzyme comprises a micellar or inverted micellar structure.

Claims 39. - 60. (cancelled).

61. (original) A method for forming an electrode for use in a biofuel cell, the method comprising

forming at least one electrical connector on a substrate;

forming at least one microchannel in a non-conductive casting mold comprised of a material that will not passivate the electrode and can be reversibly sealed to the substrate;

adhering the casting mold to the substrate;

flowing an electron conductor solution through the microchannels; and
curing the electron conductor solution to form the electrode.

62. (original) The method of claim 61, wherein the microchannels in the casting mold are formed using soft lithography.

63. (cancelled).

64. (original) The method of claim 61, wherein the substrate is flat.

65. (cancelled).

66. (original) The method of claim 61, wherein the method further comprises removing the casting mold and replacing it with a gas-permeable mold comprising larger microchannels.

67. (cancelled).

68. (original) The method of claim 66, wherein the microchannels in the gas-permeable mold are formed using soft lithography.

69. (cancelled).

70. (original) The method of claim 61, wherein the electron conductor solution comprises a carbon-based ink.

71. (cancelled).